JOURNAL OF AGRICULTURAL RESEARCH

VOL. IX WASHINGTON, D. C., JUNE 11, 1917 NO. 11

TOXICITY OF VARIOUS BENZENE DERIVATIVES TO INSECTS

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INTRODUCTION

The author in a previous paper (5)[1] pointed out the possibility of fumigating animals with nitrobenzene[2] to destroy their external parasites. In that work and in later experiments with nitrobenzene as many as 500 animals (cattle, sheep, hogs, chickens, dogs, cats, rats, and guinea pigs) have been fumigated, with only two cases of possible poisoning. One case was the fumigation of five chickens, which, through a misunderstanding of an assistant, were fumigated for 13 hours instead of 8, with the result that the chickens later died from paralysis of the central nervous system. The other instance was that of a young cat, which after one hour showed signs of uneasiness and was removed from the fumigation cage. As no symptoms of poisoning resulted in this case the cat may have been reacting to a strange environment rather than to the action of the poison.

Nevertheless, in view of the extreme toxicity of nitrobenzene as recorded in different works on toxicology, it was felt that it might be too poisonous for general use by inexperienced persons. For this reason a study of a series of benzene derivatives was undertaken with a view to determining their toxicity to insects; and from the result of this study it was hoped that one or more compounds might be found which would be quite toxic to insects while nontoxic to higher animals or plants. A study of the toxicity of the vapor of 28 benzene derivatives has been completed. A knowledge of the toxicity of the vapors of these compounds is valuable not alone for fumigation purposes but also as an

[1] Reference is made by number to "Literature cited," p. 380.

[2] In nomenclature the usage of American Chemical Society is followed.

index of their worth as contact sprays, since Shafer (6) and, more recently, McIndoo (4) have shown that most contact sprays kill by the action of their vapor rather than by the plugging of the spiracles.

COMPOUNDS USED IN THE EXPERIMENTS

From the hydrocarbon benzene C_6H_6 a great many compounds may be derived by replacement of one or more of the hydrogen atoms by certain other elements or groups of elements. These compounds are designated "mono," "di," "tri," etc., derivatives, depending on the number of hydrogens which are substituted. The following mono-substitution compounds have been tested in this study:

Benzonitrile, C_6H_5CN
Chlorbenzene, C_6H_5Cl
Brombenzene, C_6H_5Br
Iodobenzene, C_6H_5I
Phenol, C_6H_5OH
Anilin, $C_6H_5NH_2$
Benzaldehyde, C_6H_5CHO
Nitrobenzene, $C_6H_5NO_2$
Toluene, $C_6H_5CH_3$

The following di-substitution products were employed:

Xylene, $C_6H_4(CH_3)_2$ (mixture of the three possible isomeres)
Para-dichlorbenzene, $C_6H_4Cl_2$
Para-dibrombenzene, $C_6H_4Br_2$
Ortho-chlorphenol, $C_6H_4OH\ Cl$
Ortho-nitrophenol, $C_6H_4OH\ NO_2$
Salicylic aldehyde, $C_6H_4O\ H\ CHO$

Besides these di-substitution compounds, several other derivatives were used, which may be considered di-substitution compounds of benzene or mono-substitutions of toluene. They were ortho- and para-bromtoluene ($C_6H_4CH_3Br$), ortho-, meta-, and para-cresol ($C_6H_4CH_3OH$), and ortho-nitrotoluene ($C_6H_4CH_3NO_2$). Inasmuch as different compounds are obtained by substitution in the methyl group of toluene rather than in the benzene ring of toluene, two such compounds were tested: Benzyl alcohol ($C_6H_5CH_2OH$) and benzoyl chlorid ($C_6H_5CO\ Cl$). Two derivatives of xylene were tried: Bromxylene ($C_6H_3(CH_3)_2Br$) and nitroxylene ($C_6H_3(CH_3)_2NO_2$).

The xylene used in the experiments was a mixture of ortho-, meta-, and para-xylene; hence, the bromxylene and nitroxylene were also mixed compounds.

In this series is shown a wide range of compounds very different in chemical composition. A few others were tested but not included, owing to their slight volatility.

METHODS OF EXPERIMENTATION

One-liter Florence flasks of pyrex glass, closed with rubber stoppers, were used as fumigation chambers. As rubber was found to absorb the vapor of the chemicals, the stopper was coated with lead foil. Measured quantities of the compound to be tested were placed on a piece of filter paper cut just as small as possible, the paper was suspended from the

stopper inside of the flask, and the compound was allowed to evaporate. After several different insects were used in preliminary tests, the house fly (*Musca domestica* L.) was selected as being typical and easy to breed in large numbers. The flies were bred in the insectary and kept under natural conditions, thus avoiding irregular results due to the different ages and physical conditions of the wild flies. Five flies were put into each flask, the chemical introduced, and the flask tightly stoppered. When all the flies in the flask were apparently dead, they were removed to a vial and given 24 hours to revive. If none revived, the time during which the flies were exposed to the vapor was recorded. But, if the flies revived, the experiment was repeated. The average of 50 tests for a certain quantity of any chemical was found to be practically the same as the average of 5 tests; hence, in each case 5 tests were conducted. Controls showed that flies could live in a closed flask for 20 or more hours.

Since similar weights of the different chemicals do not contain the same number of molecules, and their toxicity could not, therefore, be accurately compared, it was decided to determine the toxicity in minutes for similar fractions of a gram-molecule of each chemical. Different quantities of each chemical were tested and curves plotted. As the quantity increased, it was found that each chemical had a point beyond which an increase would not give a reduction in the time required to kill. This is the point at which the air is saturated with the vapor, and differs for each chemical. As the quantity is decreased, a point is reached where the vapor is not of sufficient strength to kill. The plotted curves lie between these two points.

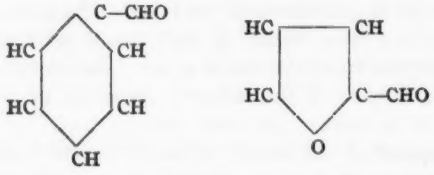
After the curves were plotted, it was found to be impossible to compare similar fractions of a gram-molecule; hence, the different fractions of a gram-molecule necessary to kill in a fixed time of 400 minutes were determined. A long period of time was selected as a more nearly correct index of toxicity. The fraction of the gram-molecule was determined by dividing the amount of the chemical necessary to kill in 400 minutes by the molecular weight of the substance. The sums given in the charts are the millionths of a gram-molecule necessary to kill five house flies in a 1-liter flask at a temperature of 70° F.

The liquid benzene compounds were measured by volume in blood-counting pipettes, and the weight of this volume was determined from the weight of 1 c. c. of the chemical. Weighed quantities of the solid benzene derivatives were dissolved in a known volume of benzene. A certain volume of this solution would contain a definite quantity of the benzene derivative. The measured volume was placed on the paper and blown for a moment to evaporate the solvent. The rapid evaporation of the solvent resulted in a lowering of the temperature, thus preventing appreciable evaporation of the compound to be tested.

RESULTS OF THE INVESTIGATION

Inasmuch as carbon bisulphid is in general use as a fumigant, its toxicity was determined for comparison with the toxicity of the benzene derivatives. Figure 1 shows curves based upon different amounts of certain of the chemicals and the time required to kill with such quantities. Owing to the extended curves of some of the substances a graphical representation was not feasible.

The data upon which the curves are based are given in Table I. Furfural is included for comparison with the aldehydes of benzene. Its relationship to benzenaldehyde is shown in the following formulas:



The results, given in millionths of a gram-molecule, are recorded in Table II and shown graphically in figure 2.

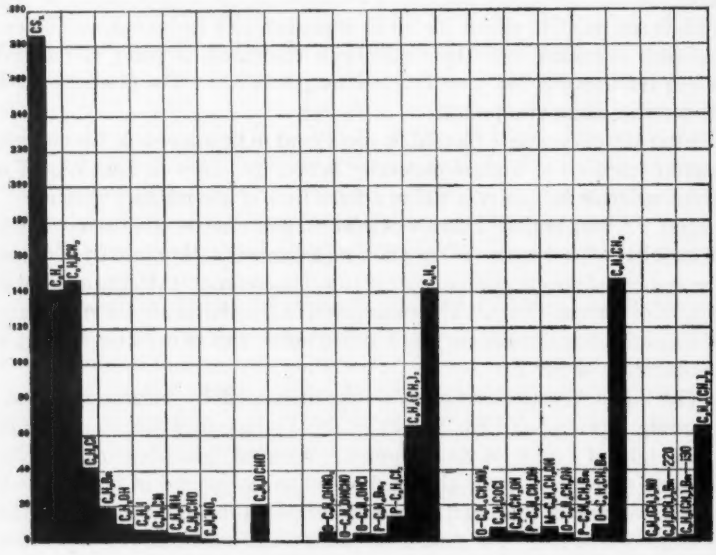


FIG. 2.—Graph showing the millionths of a gram-molecule necessary to produce the death of five house flies in a 1-liter flask at 70° F. in 400 minutes.

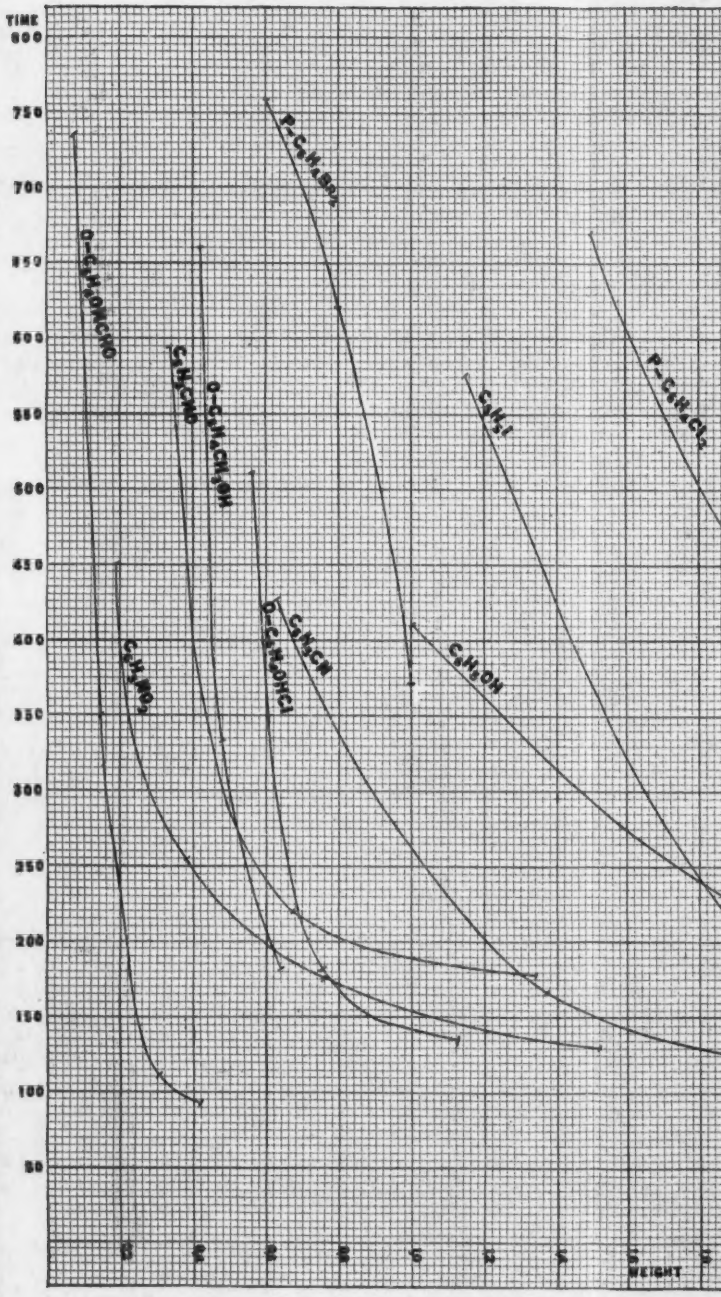
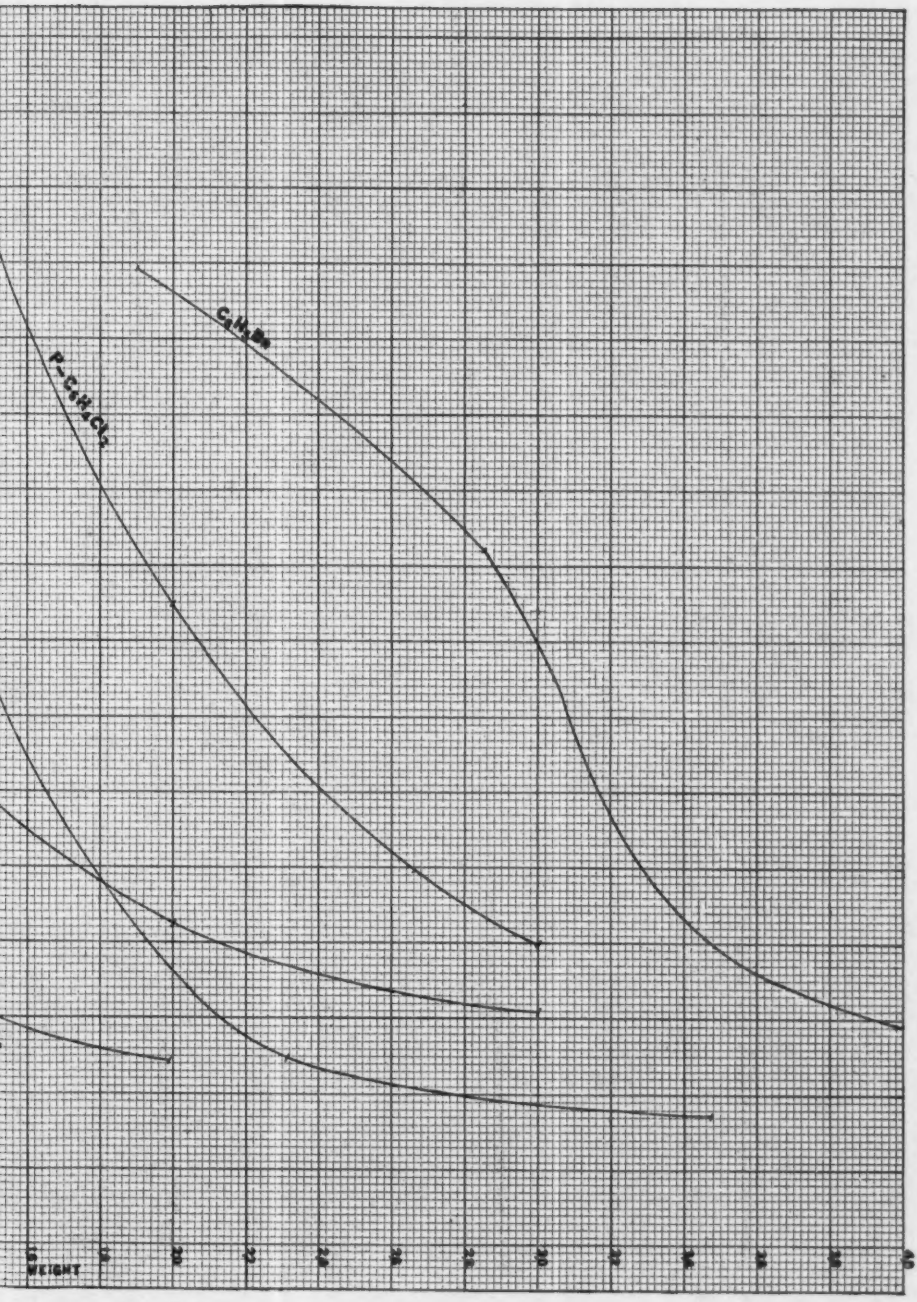


FIG. 1.—Curves showing periods of time required to l



tiine required to kill with different quantities of the different benzene derivatives.

TABLE I.—*Quantity of a chemical and time required to kill five house flies in a 1-liter flask at 70° F.*

Chemical.	Quantity of chemical required.	Time required (average of five tests).	Chemical.	Quantity of chemical required.	Time required (average of five tests).
	Gm.	*Minutes.*		*Gm.*	*Minutes.*
Carbon bisulphid	0.02179	400	Salicylic aldehyde	.00044	93
Benzene	.02028	185	Do	.00029	110
Do	.01521	349	Do	.00014	349
Do	.01015	427	Do	.00007	735
Toluene	.01548	258	Ortho-nitrophenol	.001	241
Do	.01289	498	Do	.0007	409
Do	.01032	600	Do	.0005	512
Chlorbenzene	.01265	85	Ortho-bromtoluene	.00265	159
Do	.00632	215	Do	.00177	341
Do	.00413	566	Do	.00132	610
Brombenzene	.00475	133	Para-bromtoluene	.001	318
Do	.0038	160	Do	.0005	350
Do	.0028	461	Do	.00025	351
Do	.0019	646	Do	.0001	544
Phenol	.003	155	Ortho-cresol	.00064	182
Do	.002	213	Do	.00048	333
Do	.001	411	Do	.00042	661
Iodobenzene	.00347	87	Meta-cresol	.00196	223
Do	.00231	123	Do	.00130	304
Do	.00115	576	Do	.00065	479
Benzonitrile	.00190	122	Para-cresol	.00193	249
Do	.00137	166	Do	.00129	319
Do	.00063	427	Do	.00064	360
Anilin	.00128	137	Do	.00032	435
Do	.00064	204	Benzoyl chlorid	.00257	130
Do	.00048	511	Do	.00164	273
Benzaldehyde	.00134	178	Do	.00082	486
Do	.00067	219	Ortho-nitrotoluene	.00058	281
Do	.00033	595	Do	.00036	293
Nitrobenzene	.00152	130	Do	.00029	450
Do	.00076	176	Bromxylene (B. P. 190°–250° C.)	.002	201
Do	.00038	254	Do	.001	292
Do	.00019	451	Do	.0005	487
Xylene	.01005	95	Bromxylene (B. P. 220°–250° C.)	.00087	269
Do	.00754	305	Do	.00043	356
Do	.00502	911	Do	.00022	532
Para-dichlorbenzene	.003	200	Nitroxylene	.00028	368
Do	.002	424	Do	.00014	581
Do	.001	670	Furfural	.00297	155
Para-dibrombenzene	.001	371	Do	.00223	273
Do	.0008	621	Do	.00198	437
Do	.0006	759			
Ortho-chlorphenol	.00112	131			
Do	.00075	181			
Do	.00056	512			

TABLE II.—*Quantity of a chemical necessary to kill five house flies in a 1-liter flask in an arbitrary time of 400 minutes*

Chemical.	Quantity of chemical required (in millionths of a gram-molecule).	Chemical.	Quantity of chemical required (in millionths of a gram-molecule).
Carbon bisulphid	286.3	Salicylic aldehyde	1.1
Benzene	142.3	Ortho-nitrophenol	5.6
Toluene	1475	Ortho-bromtoluene	9.4
Chlorbenzene	42.4	Para-bromtoluene	1.2
Brombenzene	19.2	Ortho-cresol	4.2
Phenol	10.8	Meta-cresol	7.9
Iodobenzene	6.6	Para-cresol	3.9
Benzonitrile	6.4	Benzyl alcohol	5.3
Anilin	5.3	Benzoyl chlorid	7.8
Benzaldehyde	3.7	Ortho-nitrotoluene	2.1
Nitrobenzene	1.8	Bromxylene (B. P. 190°–210° C.)	3.5
Xylene	6.4	Bromxylene (B. P. 220°–250° C.)	1.9
Para-dichlorbenzene	14.0	Nitroxylene	1.7
Para-dibrombenzene	4.1	Furfural	20.8
Ortho-chlorphenol	4.6		

DISCUSSION OF RESULTS

TOXICITY AND CHEMICAL COMPOSITION

By a glance at figure 2 it is noticed that all the benzene compounds used are more toxic than carbon bisulphid. The introduction of a methyl group into the benzene ring decreases its toxicity. This result agrees with the findings for higher animals of Winternitz and Hirschfelder (7) and further studies of Kline and Winternitz (3). The introduction of a halogen increases the toxicity similar to the results of Bechhold and Ehrlich (1), who found the introduction of a halogen increased the disinfection properties of phenol. This fact is true for insects whether the halogen is introduced in benzene, toluene, xylene, or phenol.

One might expect that, as toluene is less toxic than benzene, the halogen derivative of toluene would be less toxic than the similar derivative of benzene; but such does not seem to be the case. The iodin derivatives are more toxic than the corresponding bromin compounds, while both are more toxic than the corresponding chlorin derivatives. The disubstitution compounds of the halogens are more toxic than the monosubstitutions. The introduction of the cyanogen group does not increase the toxicity as much as might be supposed. The aldehyde group greatly increases the toxicity; in fact, salicylic aldehyde is the most toxic of all the compounds used in the experiments. From this result it would be expected that furfural would be much more poisonous than the results show it to be. Substitutions in the methyl group of toluene are

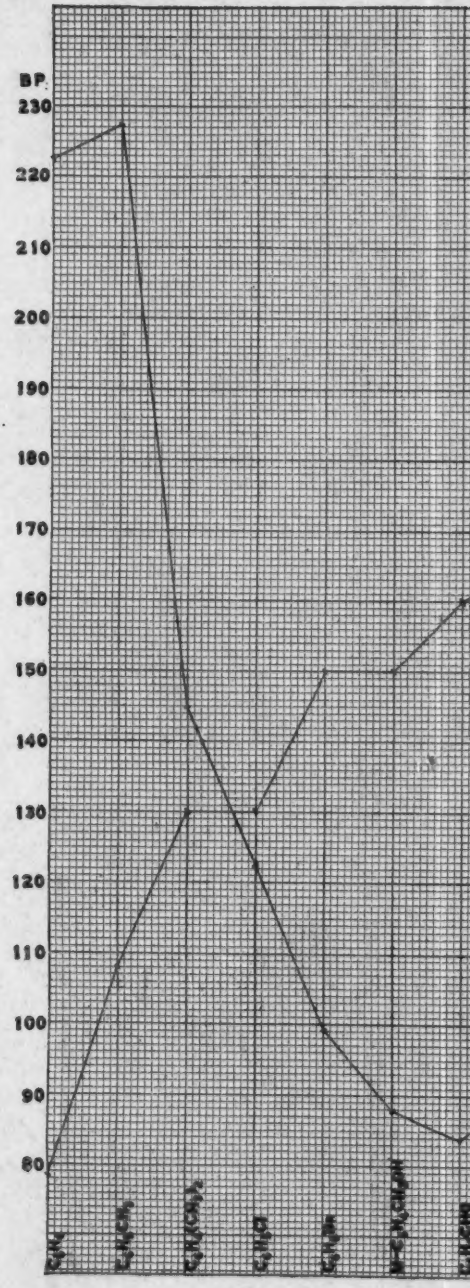


FIG. 3.—Curves showing the relationship

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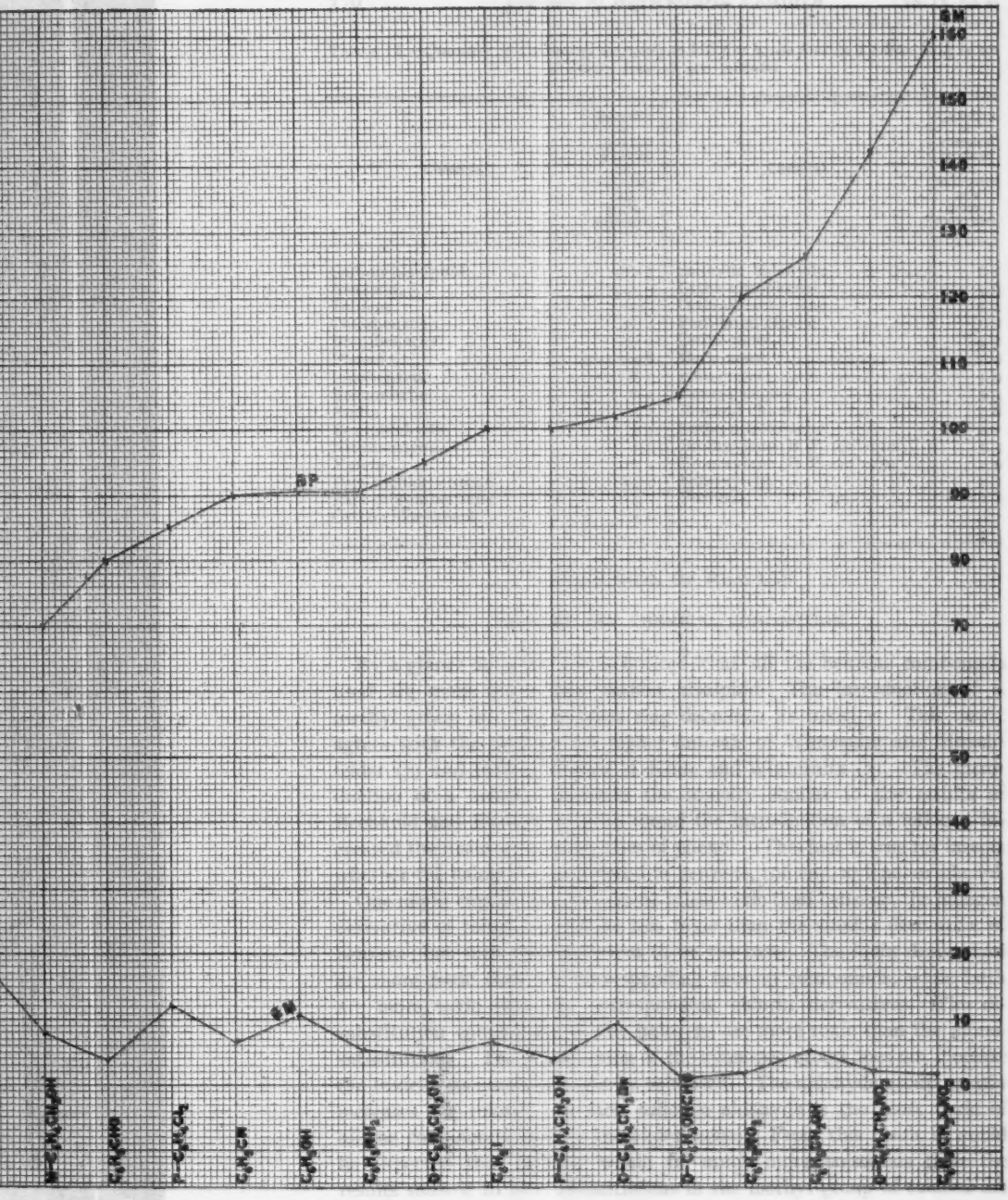

ng the relationship between boiling point (*BP*) of the compound and its toxicity expressed in millionths of a gram-molecule (*GM*).

less toxic than in the benzene ring. Para configurations seem to be more toxic than ortho configurations, while the only meta derivative tried was less toxic than either. Although certain relationships exist between chemical composition and toxicity, they are not as striking or as constant as might be expected.

BOILING POINTS AND TOXICITY

In working over the results, the author noticed a relationship between the boiling point of the chemical and its toxicity. As many of the compounds bore no guaranty of purity, the boiling points of several were determined and a curve plotted, showing the chemicals in order from the lowest boiling point to the highest. In comparison, a curve of toxicity of these compounds was plotted, as shown in figure 3. The curves show strikingly that the higher the boiling point the more toxic is the chemical. Exceptions are to be noted, which may be due to the rôle played by chemical composition in either raising or lowering the toxicity; but, in general, the curve is an increase of toxicity with an increase in the boiling point. Benzaldehyde shows a break in the curve, possibly owing to a specific action of the aldehyde. The low boiling point of furfural (96° C.) may account for its toxicity being less than would be expected from its chemical composition. Carbon bisulphid, having the lowest boiling point (47° C.), lower than any of the benzene derivatives tested, is likewise the least toxic of all the compounds.

The explanation of the relationship of boiling point to toxicity has not been ascertained. Whether the introduction of a certain element or group causes an increase in toxicity incidental to an increase in the boiling point or whether it is the relationship of boiling point to vapor pressure and volatility is not known.

BOILING POINT AND LIPOID SOLUBILITY

Another interesting observation is the relationship between boiling point and lipoid solubility. To test the lipoid solubility of the compounds, cephalin was extracted from the brain of an ox by Hirschfelder's method (2).

> Ox brain was covered with three volumes of alcohol, shaken up two or three times, and the excess of alcohol then poured off and squeezed out gently through linen, care being taken to avoid great force in wringing out the alcohol, as this tends to break up the brain tissue into very finely divided particles which pass through the filter. The residue is then covered with three volumes of ether, shaken vigorously, and filtered first through cotton and then through filter paper. The clear filtrate thus obtained is evaporated to dryness over a water bath and a yellow residue remains.

The cephalin so prepared was placed in capsule heads of 0.08 c. c. capacity and introduced into 1 c. c. of the chemical to be tested. It was found that benzene boiling at 78.5° C., toluene at 107.5° C., and xylene at 130° C. dissolved several capsules of cephalin until it finally

became thick and pastelike. By placing cephalin under a small bell jar, the air of which remained saturated with benzene, it absorbed benzene from the air until finally a liquid mass was produced. The same was true for toluene and xylene. This shows that cephalin and either benzene, toluene, or xylene are miscible in all proportions. On the other hand, brombenzene, with a boiling point of 150° C., dissolved but one capsule in 1 c. c. Benzaldehyde (165° C.) slowly penetrated the cephalin, but dissolved but little of it; while anilin (170.5° C.) salicylic aldehyde (185° C.), nitrobenzene (200° C.), and nitroxylene (240° C.) did not penetrate the cephalin and dissolved but very slight traces of it. Five c. c. of nitrobenzene, evaporated to dryness, left a very slight greasy mark on the evaporating dish. An effort was made to extract cephalin from the brain tissue with nitrobenzene without success. Lanolin also is practically insoluble in nitrobenzene. One c. c. of benzene containing 0.16 c. c. of cephalin was poured into 10 c. c. of nitrobenzene and the mixture blown with an electric fan until the benzene was evaporated, resulting in the cephalin's being thrown out of solution. From these results it appears that compounds with high boiling points are poor lipoid solvents, but are the most toxic to insects. These experiments would indicate that an increase in lipoid solubility as determined by the above method causes a decrease in toxicity in the chemicals used. Further work is now in progress to determine whether a similar relationship exists between the boiling point, lipoid solubility, and toxicity of a wider range of chemicals from the alipathic series and the terpenes.

TOXICITY OF BENZENE DERIVATIVES TO OTHER INSECTS

The toxicity of the benzene derivatives was found to be similar for other insects, and although this work has not been completed, one point may be noted. A comparison of the bluebottle fly (*Lucilia sericata* Mg.) with the house fly (*Musca domestica* L.) shows that house flies die more quickly from compounds with a low boiling point than bluebottle flies, while compounds with a high boiling point are more toxic to the bluebottle flies than to the house fly. Similarly, the cockroach (*Blatella germanica* Linn.) succumbs less readily than the potato beetle (*Leptinotarsa decemlineata* Say) to low boiling compounds and more readily to high boiling compounds. This relationship may be due to morphological differences in the insects, possibly the diameter of the spiracles or trachea.

CONCLUSIONS

Although no effort has yet been made to apply the results, certain possibilities are apparent. Even if the compounds with low boiling points are less toxic than those with high boiling points, inasmuch as more of such compounds may be evaporated before saturation is reached, better results may be obtained. This is shown in figure 4, which gives the maximum amount (in pounds) that will evaporate in 1,000 cubic feet of

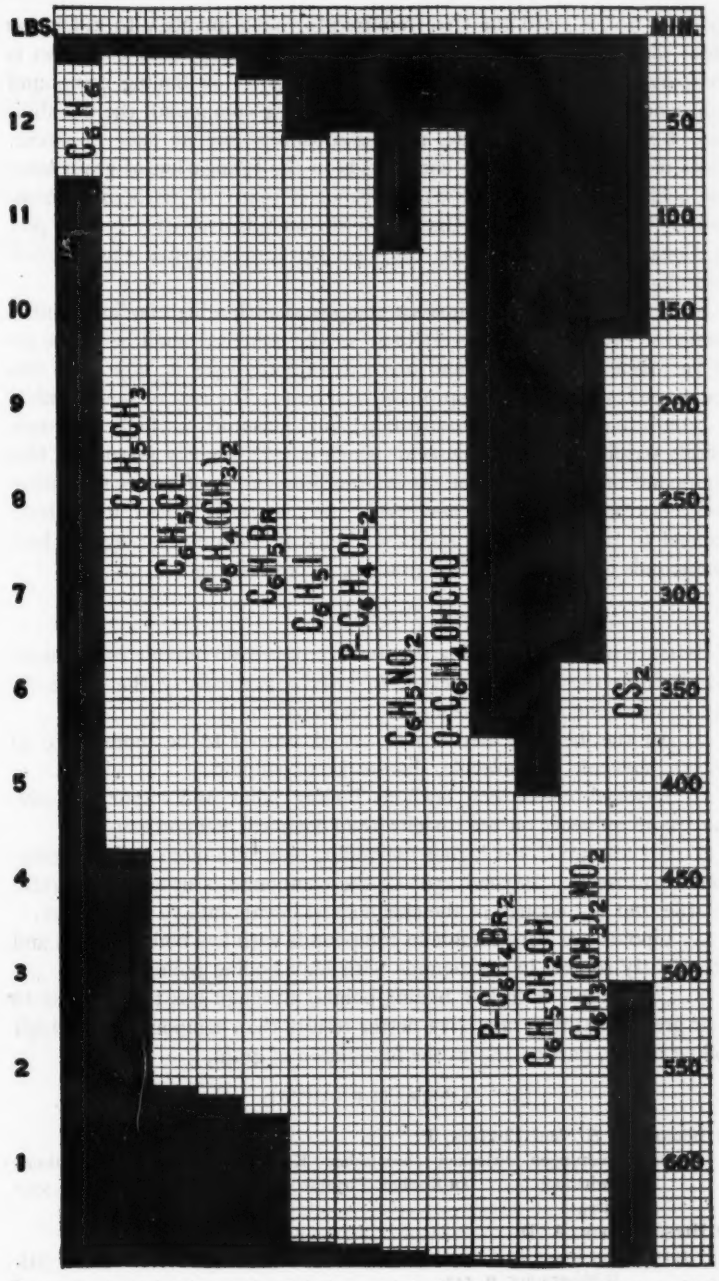

FIG. 4.—Graph showing the quantity of the benzene derivatives necessary to saturate 1,000 cubic feet of space at 70° F. and the time required by such quantity to kill house flies. Carbon bisulphid at the standard rate is given for comparison.

space at 70° F., and the time required for such quantity to kill house flies. Carbon bisulphid at the rate of 3 pounds to 1,000 cubic feet is compared with the benzene derivatives. As a low-boiling compound will penetrate grain better than a high-boiling compound, the possibilities of xylene, chlorbenzene, and brombenzene are at once apparent. Tests of the value of these compounds in the fumigation of grain have not been made. Inasmuch as the vapor of many of the benzene compounds is explosive when mixed with air, one must observe certain precautions, although in general they are far less explosive than carbon bisulphid.

For the fumigation of animals a compound with a high boiling point is needed in order that relatively little of the material shall be in the air to be taken in by the animal or to irritate the eyes or nose. In this respect salicylic aldehyde is probably the best. The cost of this chemical is prohibitive for general fumigation; but, inasmuch as higher animals readily oxidize it to salicylic acid, which is very slightly poisonous, this compound might be used for the internal fumigation of horses to destroy bots as carbon bisulphid is now used. As previously stated, it has been decided to try out a large series of chemicals before selecting the best compounds for tests as to their practicable possibilities.

SUMMARY

Data are presented showing the toxicity of certain organic compounds, mainly from the aromatic series, to insects, particularly the house fly, and certain general relationships are indicated.

(1) All the benzene derivatives tested proved to be more toxic to insects, molecule for molecule, than carbon bisulphid.

(2) Physical characters, such as boiling point and vapor pressure, have more influence on the toxicity than chemical composition.

(3) Up to 250° C. the higher the boiling point the more toxic the compound to insects. Beyond 250° C. the compound is usually so slightly volatile that not enough of the chemical will evaporate to be effective.

(4) Lipoids are very soluble in compounds with low boiling points and but slightly soluble in compounds with high boiling points.

(5) Compounds with low boiling points, although less toxic, owing to their great volatility, may give better results than compounds with high boiling points, particularly in the fumigation of grain.

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HYBRIDS OF ZEA RAMOSA AND ZEA TUNICATA

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INTRODUCTION

Data regarding the domestication of maize are still extremely meager. Yet a knowledge of this important agricultural step would be of such importance in tracing the early history of man and the beginnings of civilization that any investigations promising to throw light on the subject need no further justification.

The present study deals with the behavior of a hybrid between the two most striking variations or mutations from normal maize. Both have been considered as distinct species, *Zea tunicata* and *Zea ramosa*. Though usually referred to as agricultural species, they seem to deserve a place with the so-called species of Oenothera, which have originated through mutation.

DESCRIPTION OF PARENTS

Zea tunicata, or "pod corn," is a rather well-known variation of ordinary maize (Pl. 13, 14, 15). The most striking characteristic is that the glumes of the female inflorescence, or ear, are developed so that each seed is entirely inclosed. Associated with this character is a less conspicuous lengthening of the glumes of the staminate inflorescence that results in a thickening of the tassel (Pl. 13, *B*).

The origin of *Z. tunicata* is not known, but its occurrence in widely separated and isolated regions would indicate that it has originated independently more than once, presumably as a mutation from ordinary maize. So far as known, it has never appeared in pedigreed cultures, but there is at least one instance where it is reported to have appeared in a carefully bred commercial variety (Sconce, 1912).[1]

Z. tunicata is reported from Paraguay, Brazil, Argentina, Belgian Kongo, and many places in the United States. It was known to many tribes of American Indians. According to Parker (1910), both the Senecas and Mohawks had special names for tunicate maize, that in Seneca being translated as "original corn."

Thus far we have found no definite reference to tunicate maize in Mexico, the reference given by Sturtevant (1894) being obviously a misidentification. Neither has it been reported from Peru, and in a most extensive vocabulary of the Quichua terms relating to maize obtained in Peru by Mr. O. F. Cook, of the Bureau of Plant Industry, who made

[1] Bibliographic citations in parentheses refer to "Literature cited," p. 395.

special inquiry for this type of maize among well-informed people familiar with Indian agriculture, there is no mention of tunicate maize.

In hybrids with nontunicate varieties the tunicate character behaves as a dominant, but in our experiments we have never been able to secure a homozygous tunicate strain. Progenies resulting from the selfing of tunicate plants have, with us, always shown segregation into approximately three tunicate plants to one normal.[1]

The tunicate plants in self-pollinated progenies are separable into two classes, one producing typical tunicate ears and thickened tassels like the parent plant (Pl. 13, *B;* 14, *B*), the other with greatly enlarged tassels containing both staminate and pistillate flowers, and with the ear either aborted or bearing greatly enlarged and usually sterile spikelets (Pl. 13, *A;* 14, *B;* 15). This last class represents approximately one-third of the tunicate plants. Although these plants produce what appears to be normal pollen in the terminal inflorescence, the long glumes never open and the pollen is not shed; and we have not been successful in securing selfed seed of this form.

The ratios in which the different classes occur would indicate that the class with the bisexual terminal inflorescence is the homozygous form and that the ordinary tunicate plants represent the heterozygous form, a cross between the form with the bisexual inflorescence and the normal nontunicate maize. If the ordinary tunicate type can occur in a homozygous form, we should expect one in four of the plants grown from a self-pollinated tunicate plant to be homozygous, and the progeny of such homozygous plants should be all podded, whether cross or self-pollinated. This has not been the case in our experiments. If only a few progenies were grown, the failure to secure an all-tunicate progeny might, of course, be ascribed to the accidental selection of heterozygous instead of homozygous parents.

In the course of our experiments the progenies of 43 different tunicate plants have been grown and in all of these progenies, except one, nontunicate plants appeared. The one exception produced only eight plants. And, since only two or three normal plants were expected, it is not surprising that none appeared. Of the remaining 42 parent plants, 14 might have been expected to prove homozygous. That none proved to be homozygous can hardly be accidental, since the chances against it are over 400,000 to 1. It is therefore concluded that, in the material that has come under our observation, the ordinary type of tunicate plants represents a case of imperfect dominance, and that it is unfixable,

[1] A comparison of the ratios of tunicate to nontunicate plants shows the following:

	Tunicate.		Nontunicate.
Self and tunicate×tunicate	288		94
Expected	286.5	±5.7	95.5
Tunicate×nontunicate	99		117
Expected	108	±5.0	108

like the Andalusian fowls. Our experiments have contained tunicate strains from three distinct sources; but, since other workers report the existence of pure-tunicate strains, it may be that still other stocks behave differently.

The distinction between full tunicate and half tunicate has not always been made in our pedigrees, but the records show 17 progenies where the number of full-tunicate plants is recorded. The total number of plants in these 17 progenies is 187, of which 46 were classed as full tunicate. If, as suggested, the full-tunicate plants are the homozygous form, the expected for the number of individuals involved would be 62, a deviation of 16, or four times the probable error, a rather large deviation to be ascribed to chance, but not sufficiently aberrant to offset the failure to secure homozygous individuals among the half-tunicate plants. The distinction between full and half tunicate is not always easy to make, and it would appear from the ratios that we have been referring some of the less pronounced examples of the full tunicate to the half-tunicate class. By concentrating selection on this group of plants, more or less intermediate between full and half tunicate, it may be possible to secure a homozygous strain. But in the stocks with which we have been experimenting, individuals of the type shown in Plate 13, *A*, or that shown by East and Hayes (1911) would not serve as examples of pure-tunicate maize.

The class with bisexual terminal inflorescence, which is here assumed to be homozygous, will be referred to as "full tunicate" and the ordinary tunicate type, which we look upon as heterozygous, will be termed "half tunicate." The term "tunicate," or podded, will be used as a general term including both of the above classes.

Z. ramosa, or branched maize, is a variation from ordinary maize discovered by Dr. W. B. Gernert (1912) at the Illinois Agricultural Experiment Station. The original ear was found in 1909 in a field of Leaming corn.

Z. ramosa differs from the normal maize in having the pistillate inflorescence, or ear, which is normally simple, replaced by a compound inflorescence branched like the tassel (Pl. 14, *A*, *c*). There is also a less striking but equally significant change in the branching of the tassel (Pl. 16). In normal maize the terminal inflorescence bears a number of branches at its base. Above the uppermost branch the axis is continued into what is termed the "central spike," where the pairs of spikelets are borne directly on the main axis of the inflorescence. Thus, in passing from the base to the tip of the tassel, there is an abrupt transition from the uppermost branch to simple pairs of spikelets. In the *Z. ramosa* tassel the branches are much more numerous and gradually decrease in size from the base upward, the transition from branches to pairs of spikelets being imperceptibly gradual.

Unlike *Z. tunicata*, *Z. ramosa* is a recessive variation. The dominance of normal maize over this variation seems complete. We have never been able in any way to distinguish between plants heterozygous for the *ramosa* character and normal maize. So far as observed, the character behaves as a simple Mendelian unit.

Krafft (1870) described and figured a branch pistillate inflorescence that appears to have been of the same character as *Z. ramosa*. The reference is of interest as indicating that *Z. ramosa*, as well as *Z. tunicata*, may be looked upon as having originated more than once.

NATURE OF THE VARIATIONS

In normal maize one of the characters differentiating the male and female inflorescence is that the glumes are greatly reduced in the pistillate inflorescence, so that the kernels are naked on the cob. In *Z. tunicata* this differentiation is lost, and the kernels are inclosed in glumes as long as those of the staminate inflorescence.

Another specialized character of normal varieties of maize is the suppression of the branches in the pistillate inflorescence, or ear. In *Z. ramosa* this specialization is lost, and the ear is as completely branched as the tassel. A further loss of specialization in *Z. ramosa* is that the differentiation between branches and pairs of spikelets is lost, the one grading into the other in both ear and tassel.

In considering these losses of specialization it is desirable to keep in mind the fact that in *Z. tunicata*, as in ordinary maize, the ear is a pistillate homologue of the central spike of the tassel, while in *Z. ramosa* the ear is a pistillate replica of the entire tassel. The loss of specialization in *Z. tunicata* affects the characters of the floral organs and spikelets, while in *Z. ramosa* the general form of the pistillate inflorescence is changed to conform to that of the tassel.

Both *Z. ramosa* and *Z. tunicata* are variations from normal maize toward the general type of grasses, and as such may be looked upon as reversions, since both cases involve a loss of a specialization that distinguishes maize from practically all other grasses.

Recent investigations have shown that many reversions may be explained as recombinations, but neither *Z. ramosa* nor *Z. tunicata* results from the recombination of latent factors, or cryptomeres. Had the first appearance of *Z. ramosa* and *Z. tunicata* been the result of the fortuitous combination of factors, or cryptomeres, it would seem to follow that when the new combination was crossed with the parent stock these factors should have been again redistributed and the combination necessary to bring the new characters again into expression should have occurred much less frequently than the observed one in four individuals.

Both of these variations may also be considered either as examples of homœosis (Leavitt, 1909) or as metaphanic variations (Cook, 1915).

The transference of long glumes and the branched conditions from tassel to ear might be looked upon as excellent examples of homœosis. There is, furthermore, a large series of abnormalities that occur in the terminal inflorescence of full-tunicate plants that lend themselves to this interpretation. Spikelets develop into branches, glumes develop into pistils, lodicules develop into glumes, and so on.

On the other hand, it is difficult to think of the nicely graded length of the branches of the tassel of *Z. ramosa* as a translocation of characters. This character is better explained as a metaphanic variation or loss of differentiation, an explanation that would also apply to the branched ear of *Z. ramosa* and the lengthened glumes on the ears of *Z. tunicata*. But here the variation rather overshoots the mark; for, instead of the glumes of the ear being intermediate in length between glumes normal to the ear and the tassel, the glumes of the tunicate ear much exceed normal tassel glumes, and the tassel glumes are themselves much elongated. Neither do the other abnormalities of *Z. tunicata* appear to be typical metaphanic variations, since many of them are repetitions and accentuations rather than intermediate expressions.

There is a sense in which homœotic as well as metaphanic variations may be viewed as a loss or partial loss of differentiation. In typical metaphanic variations the normal specialization of parts is replaced by organs of intermediate form that may be taken to represent an unspecialized ancestral condition. In homœosis instead of the normal specialization certain cells exhibit the characters and potentialities of cells belonging to an entirely different part of the individual. In either instance this loss of specialization or the more or less complete return of cells to an earlier and less specialized condition may be viewed as a reversion.

After concluding that *Z. ramosa* and *Z. tunicata* may be classed as reversions, it is still possible to look upon them as mutations. Both are wide departures from the parent type, and the evidence is that both attained this departure at one step, so far as visible variations are concerned.

With *Z. ramosa* the case is simple. It is a recessive mutation and the dominance of normal maize is complete. The inheritance of *Z. tunicata* is somewhat more complicated. If the interpretation advanced above is correct, and the homozygous form is practically sterile and the common forms heterozygous, *Z. tunicata* would constitute a dominant mutation in which the dominance is imperfect.

The characters separating the two mutations are sharply contrasted. In *Z. ramosa* there is no tendency for the glumes to be elongated more than in normal maize. In tunicate maize the branches of the tassel are no more numerous than in normal maize; and, although the spikelets are much enlarged, the transition from branches to pairs of spikelets is as abrupt as in normal maize. When ears develop on full-tunicate plants, there may be branches near the base of the ear, but these are not at all

homologous to the branches of *Z. ramosa*. The branches in the ear of *Z. tunicata* are proliferated spikelets, while the branches of a *ramosa* ear are, like the branches of the tassel, divisions of the main axis with no evidence of a subtending bract.

DESCRIPTION OF THE HYBRID

The cross between *Z. tunicata* and *Z. ramosa* was made at Lanham, Md., in 1914. The female parent was a plant of *Z. ramosa* grown from seed supplied the Department of Agriculture by Dr. Gernert. The male parent was a plant of a tunicate strain developed by Mr. H. J. Sconce. The parent ear was what is here designated as half tunicate.

Nine first-generation plants of this cross were grown at Chula Vista, Cal., in 1915. Of these, four were tunicate and five normal, indicating the heterozygous nature of the half-tunicate parent plant. The tunicate plants were all half tunicate, and no trace of the *ramosa* characters could be seen.

Five self-pollinated first-generation ears were selected for planting in 1916. Three of these ears were tunicate and two normal. The three tunicate ears all showed about the same development of the tunicate character. The seeds were all well covered by the glumes, but the longest glumes did not exceed 30 mm.

The second generation was grown in 1916 at Lanham, Md. Four hundred and eight plants matured, 326 from the three tunicate ears, and 82 from the two nontunicate ears of the first generation.

The progeny of the nontunicate or normal F_1 plants may be dismissed with the statement that the F_2 plants showed segregation into normal and *ramosa* in the ratio of 3 to 1, the numbers being 65 normal and 17 *ramosa*. In none of these plants was there evidence of tunicate characters.

The first impression to be gained from the descendants of the tunicate plants as they came into flower was that there was a completely heterogeneous mixture of the characters of the two parents, together with many new monstrosities. On closer examination it soon became evident that there was one clearly defined group of plants having all the characters of normal maize. It was also possible to distinguish many plants with characteristic *tunicata* or *ramosa* tassels. Among the latter types, however, there were many intermediates, and in addition there was an entirely new type of inflorescence. In this new type the branching habit was developed to a grotesque extreme. As soon as branches formed these again branched. This division continued until the end of the growing season when the tissue was still in an embryonic condition, and nothing resembling floral or foliar organs was formed. The result was a white succulent mass (Pl. 17, 18). This peculiar formation occurred in both lateral and terminal inflorescences, though it was much more common in the former, and in terminal inflorescences it was usually confined to the basal branches.

This abnormality is similar, if not identical, with an abnormality discovered by Blaringhem (1907) in a strain of *Z. tunicata* and termed by him "cauliflower." To judge from Blaringhem's description and plates, the chief differences between his specimens and ours—and these may be only of degree—are that in his examples the disturbance did not extend to the entire inflorescence, and the ultimate ramification terminated in microscopic floral organs, while in ours no sign of floral organs was developed.

Before an examination of the pistillate inflorescences was possible the growing plants were numbered and classified with respect to the character of the tassel. The classification was made on the general appearance of the tassel, and the following classes were recognized: Normal, half tunicate, full tunicate, *ramosa*, and *tunicata-ramosa*, the last class comprising those plants in which both *tunicata* and *ramosa* characters could be recognized, and frequently with more or less tendency to "cauliflower."

There was no occasion for doubt regarding the plants referred to the normal class. The presence of the *tunicata* character was also unmistakable, but the distinction between half and full tunicate was not always easy to make. In the whole field there were three plants recorded as intermediate between half and full tunicate. The plants referred to *ramosa* formed a fairly distinct class, though it was evident that in many of the plants the tassels were more dense with longer glumes and more nearly pendent than was normal to pure *ramosa*, suggesting the presence of *tunicata* characters. There was, thus, some intergradation between the *ramosa* plants and those classed as *tunicata-ramosa*. This uncertainty was dispelled when the ears were later examined, the *ramosa* and *tunicata-ramosa* classes proving to be completely discontinuous.

GAMETIC COMPOSITION

The numbers in which the various classes of plants occur are capable of explanation by the assumption of a comparatively simple gametic composition. The terminology here used is to assign a letter to the dominant member of each allelomorph and the same letter, primed, to the recessive member. To those who are accustomed to the presence and absence method of notation it will only be necessary to look upon the primed letters as the absence of the factor, usually designated by a lower-case letter. The custom followed by many workers of assigning the unmodified letter to the factor as it exists in the wild or unmutated form is impracticable in agricultural plants. Since we have not this base line, the unmodified letter is assigned to the dominant member.

On this basis, beginning with the dominant form, full-tunicate plants may be assigned the gametic composition $TTRR$, T representing the tunicate factor and R the dominant allelomorph to the *ramosa* factor. *Ramosa*, the other parent, would then be $T'T'R'R'$. Ordinary maize with

respect to these characters would be $T'T'RR$. Half-tunicate plants, such as the male parent of the hybrid, would be $TT'RR$. In a cross between such a plant and *ramosa* the first generation would consist of two kinds of plants, $TT'RR'$ and $T'T'RR'$. Since all contain R, which is dominant, none would be *ramosa*. One half being heterozygous, for T would be half-tunicate, the other half would be nontunicate or normal, being homozygous for T'. Since the tunicate plants of the first generation are heterozygous for both T and R, the selfing of such individuals would give all possible combinations (Table I).

TABLE I.—*Gametic composition of* the *hybrid between Zea ramosa and Zea tunicata*

PARENTS	*Zea tunicata.*	*Zea ramosa.*
P_1 Gametes	TR $T'R$	$T'R'$
F_1 Zygotes	Half tunicate	Normal
Gametes	TR, TR', $T'R$, $T'R'$	$T'R$, $T'R'$

F_2	TR	TR'	$T'R$	$T'R'$
TR	$\frac{TR}{TR}$ Full tunicate	$\frac{TR'}{TR}$ Full tunicate	$\frac{T'R}{TR}$ Half tunicate	$\frac{T'R'}{TR}$ Half tunicate
TR'	$\frac{TR}{TR'}$ Full tunicate	$\frac{TR'}{TR'}$ *Tunicata-ramosa*	$\frac{T'R}{TR'}$ Half tunicate	$\frac{T'R'}{TR'}$ *Tunicata-ramosa*
$T'R$	$\frac{TR}{T'R}$ Half tunicate	$\frac{TR'}{T'R}$ Half tunicate	$\frac{T'R}{T'R}$ Normal	$\frac{T'R'}{T'R}$ Normal
$T'R'$	$\frac{TR}{T'R'}$ Half tunicate	$\frac{TR'}{T'R'}$ *Tunicata-ramosa*	$\frac{T'R}{T'R'}$ Normal	$\frac{T'R'}{T'R'}$ *Ramosa*

F_2	$T'R$	$T'R'$
$T'R$	$\frac{T'R}{T'R}$ Normal	$\frac{T'R'}{T'R}$ Normal
$T'R'$	$\frac{T'R}{T'R'}$ Normal	$\frac{T'R'}{T'R'}$ *Ramosa*

From the above hypothesis and the behavior of the first generation, we should assume that in F_2 all plants homozygous for R' would be *ramosa*. All plants either heterozygous or homozygous for R and homozygous for T' would be normal. Those heterozygous for T and with at least one R would be half tunicate. Those homozygous for T and with at least one R would be full tunicate. Since the above conditions are not mutually exclusive, there would be combinations calling for the plants to be *ramosa* and at the same time either full or half tunicate.

There were 326 second-generation plants from the *Z. tunicata* first-generation ears. The observed number compared with the number expected in accordance with the gametic composition assumed above are given in Table II.

TABLE II.—*Comparison of observed and expected ratios of the different classes of plants*

Number expected out of each 16.	Gametic composition.	Characters of plant.	Expected number.	Observed number.
1	$T'T'RR$	Normal	61.2	64
2	$T'T'RR'$	do		
2	$TT'RR$	Half tunicate	122.0	121
4	$TT'RR'$	do		
1	$TTRR$	Full tunicate	61.2	61
2	$TTRR'$	do		
1	$TTR'R'$	*Tunicata-ramosa*	61.2	64
2	$TT'R'R'$	do		
1	$T'T'R'R'$	*Ramosa*	20.4	16
Total 16			326.0	326

When the character of the ear was considered, all the groups, with the exception of half and full tunicate, were perfectly distinct, with no doubtful individuals.

CORRELATIONS BETWEEN TYPE OF PISTILLATE AND STAMINATE INFLORESCENCES

The degree of correlation between the characters of the staminate and pistillate inflorescences may be judged from an examination of Table III.

TABLE III.—*Characters of the staminate and pistillate inflorescences of F_2 plants of Zea ramosa × Zea tunicata hybrid*

Character of pistillate inflorescence.	Character of staminate inflorescence.						
	Normal.	Half tunicate, glumes 12–24 mm.	Full tunicate, glumes above 25 mm.	*Ramosa.*	*Tunicata-ramosa.*	Staminate inflorescences destroyed.	Total.
Normal glumes, 5 mm	64						64
Half tunicate, glumes 10 to 44 mm		120	3				123
Full tunicate, glumes above 45 mm. or earless		4	49			6	59
Ramosa				16			16
Tunicata-ramosa				37	23	4	64
Total							326

NORMAL PLANTS.—It will be seen that among normal plants the correlation is perfect. All plants classed as normal by the tassels proved to have normal ears, and vice versa. The number of normal plants was 64; the expected number 61.2.

HALF-TUNICATE PLANTS.—As previously stated, the distinction between half- and full-tunicate plants is not sharp. If one relies on the gen-

eral appearance, there is seldom any doubt regarding the class to which a plant should be referred; but when the differences are formulated, there is some overlapping. The most obvious tassel characters are the long glumes, the pendent tassel, and the presence of pistillate flowers. Of these, the length of glumes appeared to be the most significant, and was the only one systematically recorded. When the glumes are over 25 mm. long, the tassel in most instances obviously belongs to the full-tunicate class. In classifying the ears, the length of the glumes is also the best character. The dividing line here falls on 45 mm.

There were but three half-tunicate plants in which the length of the staminate glumes exceeded 24 mm. There was, however, no perceptible correlation between the length of glumes in the male and female inflorescences among the half-tunicate plants. One hundred and twenty-three plants were referred to this class. The expected was 122. The nearest approach to an intermediate between half-tunicate and normal plants is shown in Plate 19.

FULL-TUNICATE PLANTS.—Fifty-three plants were classed as full tunicate. Of these, 12 produced no ear. Of the remainder, all but 4 had staminate glumes at least 25 mm. long. There is, then, almost a perfect correlation between the type of tassel and the type of the ear; but here again there is no correlation inside the group.

Even the group of plants that were earless did not differ from the plants with ears with respect to the length of the staminate glumes.

There were, in addition, 6 full-tunicate plants with tassels destroyed through accident, making a total of 59 plants in this class. The expected number was 61.2.

RAMOSA PLANTS.—Sixteen plants with pure *ramosa* ears all had *ramosa* tassels. The expected number was 20.4.

TUNICATA-RAMOSA PLANTS.—There were 60 plants that exhibited both *ramosa* and *tunicata* characters. Of these, 23 exhibited the characters of both parents in the tassel as well as the ear. The remaining 37 plants all had *ramosa* tassels in which no tunicate characters were obvious (Pl. 20, *A*).

Of the 23 plants which exhibited both *ramosa* and *tunicata* characters in the tassel, 19 had cauliflower ears (Pl. 20, *B*), 3 showed mixtures of cauliflower and tunicate tendencies, and 1 produced no ear.

In the 37 plants which showed no tunicate character in the tassel, 2 produced cauliflower ears, 11 showed mixtures of cauliflower and tunicate, and 24 were both branched and tunicate without cauliflower (Pl. 21).

In addition to the above, there were 4 plants in which the tassels were accidentally destroyed, making a total of 64 plants in the group. The expected number was 61.2. The nature of the plants combining the characters of both parents is shown in Table IV.

TABLE IV.—*Characters of staminate and pistillate inflorescences of the tunicata-ramosa group of F_2 plants of Zea ramosa × Zea tunicata hybrid*

Character of pistillate inflorescence.	Character of staminate inflorescence.				
	Ramosa.	Without cauliflower.	Partial cauliflower.	Completely cauliflower.	Total.
Ramosa					
Without cauliflower	24				24
Partial cauliflower	11		3		14
Completely cauliflower	2		19		21
Aborted			1		1
Total	37		23		60

If the second-generation plants are examined for each of the parental types separately, there is seen to have been a simple 1 to 3 segregation in both instances. One-fourth of the total number of plants are *ramosa* and three-fourths non-*ramosa* (observed, 79 to 247; expected, 81.5 to 244.5). One-fourth are nontunicate and three-fourths tunicate (observed, 80 to 246; expected, 81.5 to 244.5). The distinction between half and full tunicate could not be made when these characters were combined with the *ramosa* character. The various combinations of parental characters, occurring as they do in the normal dihybrid ratios, show that the *tunicata* and *ramosa* characters are not genetically correlated.

In addition to the notes on the inflorescences, the height of each plant was recorded. From these measurements it develops that there were consistent differences in the height of the segregated groups. The averages are given below:

Type of plant.	Height (cm).
Normal	221±2.7
Ramosa	195±2.2
Half tunicate	195±2.4
Tunicata-ramosa	191±2.6
Full tunicate	171±2.7

These differences in height can hardly be explained as differences in vigor due to different degrees of heterozygosity of the characters concerned, since the *ramosa* and half-tunicate groups, which are of the same and an intermediate height, are at once the least and most heterozygous of all the groups.

ORIGIN AND SIGNIFICANCE OF CAULIFLOWER INFLORESCENCE

In our experiments the appearance of cauliflower in the inflorescence seems definitely confined to plants in which both the *tunicata* and *ramosa* characters are, as it were, endeavoring to come into expression—that is,

to the plants homozygous for the recessive *ramosa* character and either homozygous or heterozygous for the dominant tunicate character.

The intimate relation between the *ramosa* characters and the cauliflower type of inflorescence may have a bearing on the appearance of cauliflower in Blaringhem's strain of *Z. tunicata*. If the two abnormalities are really of the same nature, the possibility is suggested that in Blaringhem's strain there was a re-occurrence of the *ramosa* mutation. Blaringhem had but few plants of this strain under observation; hence, the absence of pure *ramosa* plants would not be remarkable. In the course of our experiments hundreds of plants of *Z. tunicata* have been examined and their abnormalities studied, but nothing resembling the cauliflower type of inflorescence has been found. In the hybrid under discussion, cauliflower is more definitely associated with the *ramosa* than with the *tunicata* characters. The phenomenon itself appears as an accentuation of the branched habit, and while plants with cauliflower ears occurred without exhibiting *tunicata* characters in the tassel, the *ramosa* characters are in all instances fully expressed.

In making this cross between these two variations from normal maize, each of which may be looked upon as a reversion, the hope was entertained that their combination might bring to light still other ancestral characters and help to give us a slightly more definite conception of the ancestors of maize. In this hope of securing direct evidence we were disappointed. When the two characters are forced to come into expression in the same individual, the result is either a mixture of the two characters or a sterile monstrosity which by no stretch of the imagination can be regarded as an ancestral condition.

From the fact that *Z. tunicata* is a dominant variation Blaringhem (1907) concludes that it is a new or progressive mutation without significance in the study of the ancestry of maize. Since in every particular by which *Z. tunicata* departs from normal maize, it does so by replacing the specialized characters of maize with characters common to practically all other grasses, to place so much emphasis on the dominance of the character seems unreasonable.

The phylogenetic bearing of *Z. ramosa* is less obvious, but even here it seems not unwarranted to consider the variation in the nature of a reversion. The partial incompatibility of the two variations may be explained on the assumption that they represent the recurrence of characters from two widely separated ancestors.

SUMMARY

Z. ramosa and *Z. tunicata* are looked upon as mutative reversions, the one recessive, the other dominant, as compared with normal maize. The result of crossing these two mutants has been to show that both behave as independent Mendelian units. In the second generation there appears (1) normal maize showing none of the characters of either muta-

tion, (2) the recurrence of both parental types in an apparently pure form, and (3) plants combining the characters of both the mutations. In the last group normal expression is inhibited, and the result is frequently the appearance of a totally different type of inflorescence called "cauliflower," which is sterile, the character being abnormal to the extent that the tissue remains in an embryonic condition, the result being a much-branched, white, succulent mass.

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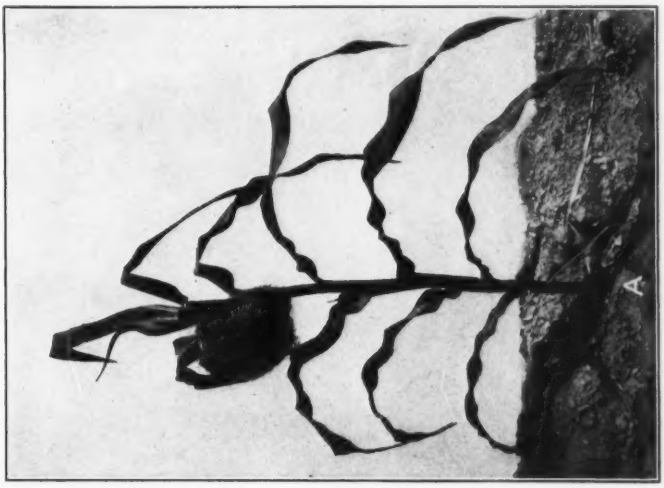
PLATE 13

Zea tunicata:

A.—Plant of full-tunicate type.
B.—Plant of half-tunicate type.

B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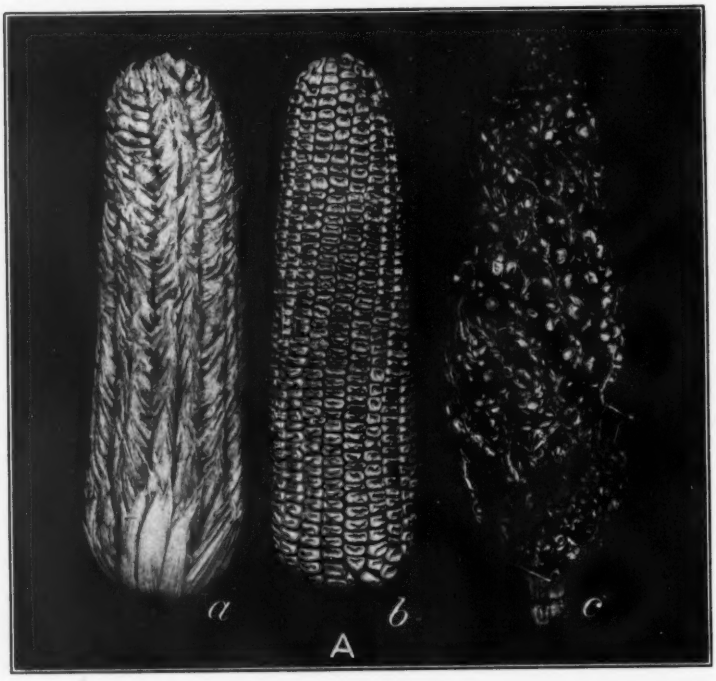

a
b
c
A

a
B
b

PLATE 14

A.—Pistillate inflorescence of maize. *a*, *Zea tunicata:* Half-tunicate type. *b*, Normal maize. *c*, *Zea ramosa.*

B.—*Zea tunicata:* Terminal and lateral inflorescence. *a*, Full-tunicate type; *b*, half-tunicate type.

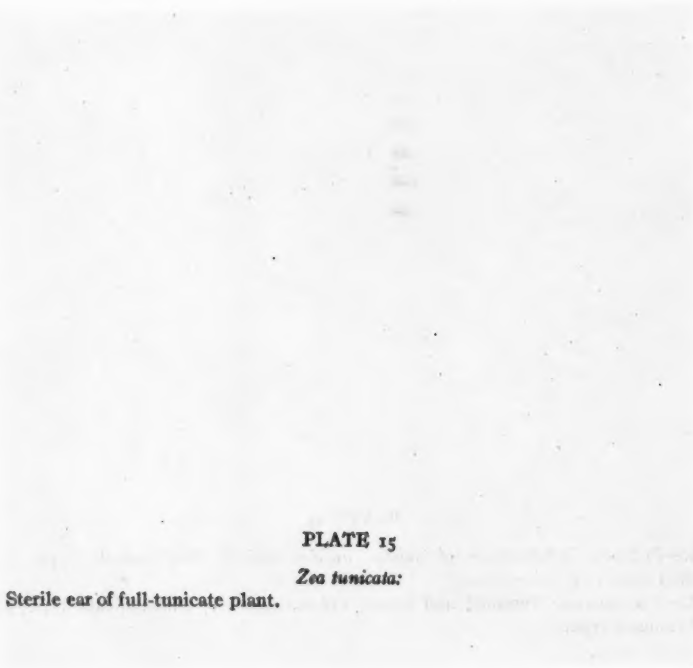


PLATE 15

Zea tunicata:

Sterile ear of full-tunicate plant.

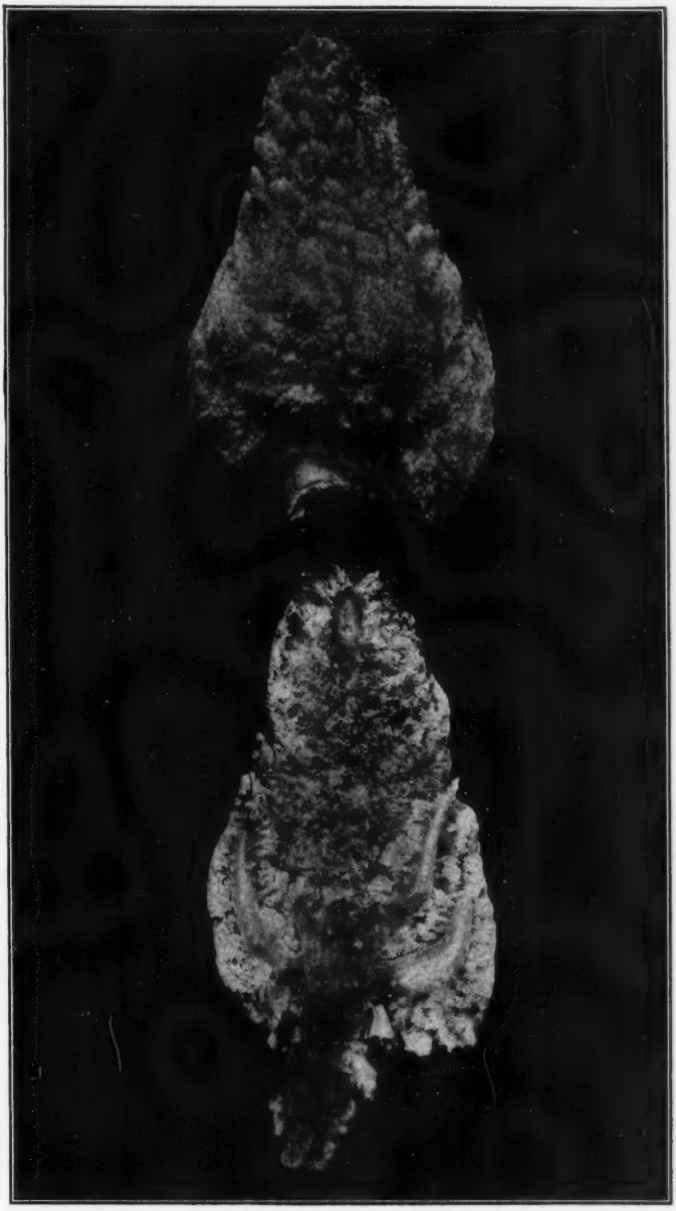
PLATE 16

Zea ramosa:

Staminate and pistillate inflorescence.

PLATE 17

"Cauliflower" lateral inflorescence borne on F_2 plants of *Zea ramosa* × *Zea tunicata* hybrid.



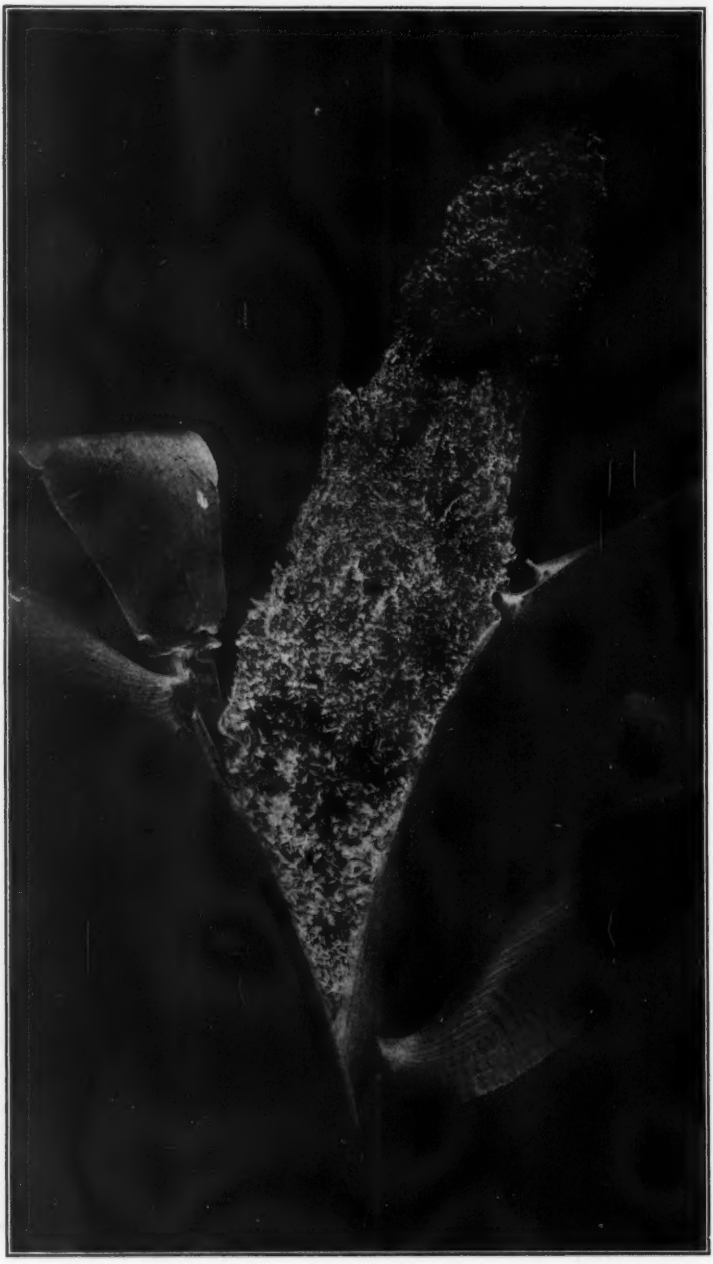
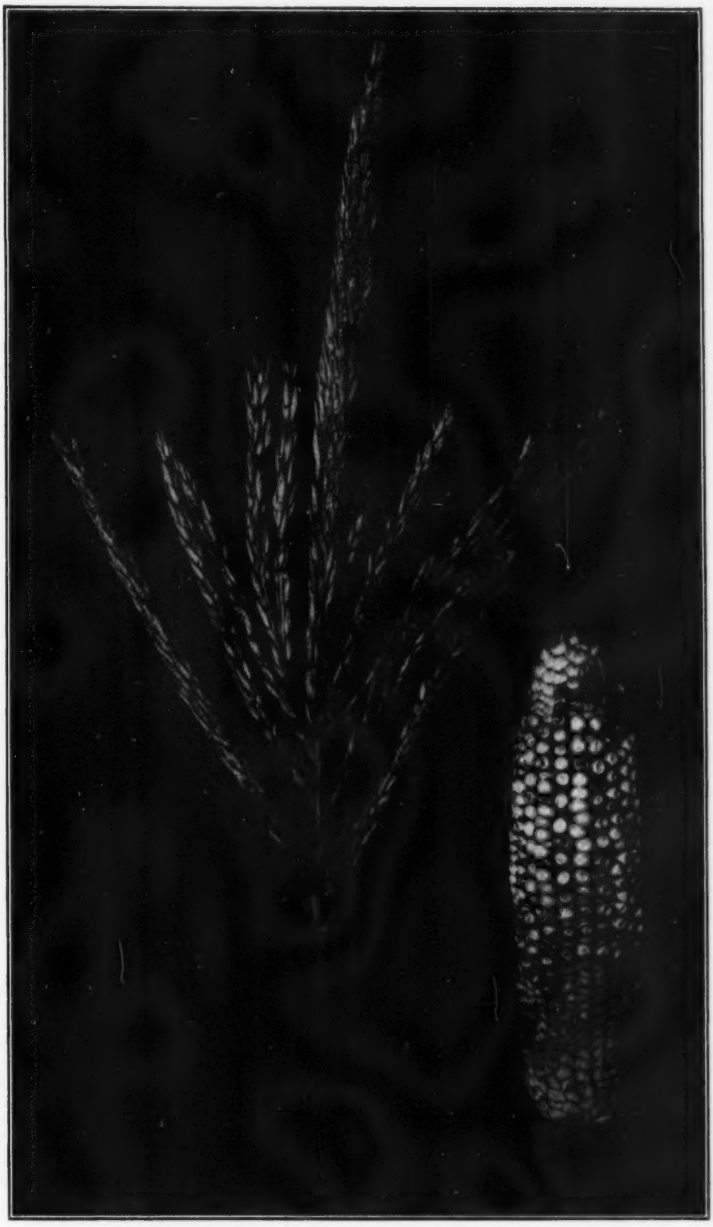


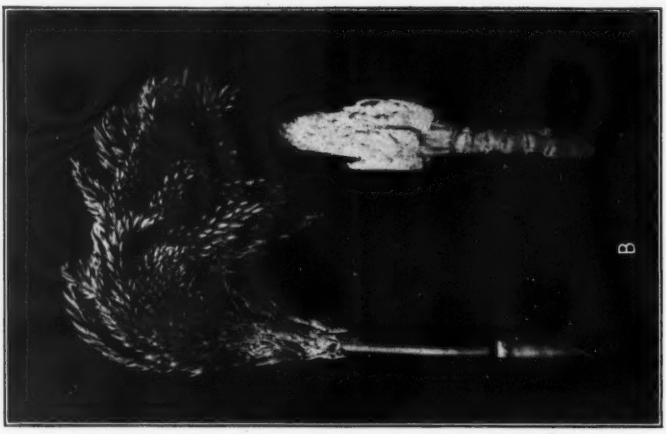
PLATE 18

Terminal inflorescence, cauliflower type of F_2 plant of *Zea ramosa* × *Zea tunicata* hybrid.

PLATE 19

Half-tunicate F_2 plant, the nearest approach found to an intermediate between normal and half tunicate.




B

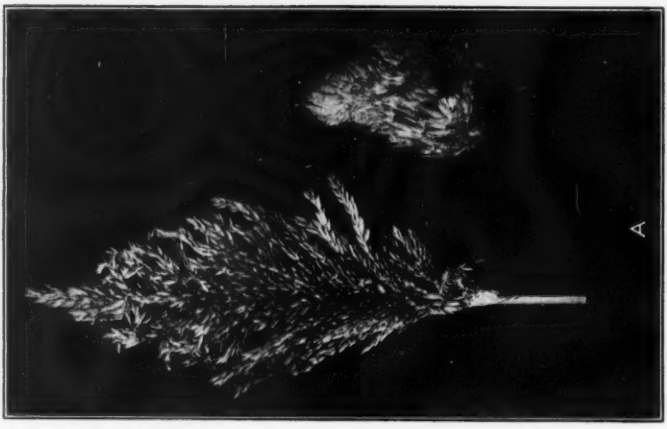

A

PLATE 20

A.—Terminal and lateral inflorescence of F_2 plant, showing the ear both branched and tunicate, and the tassel with only *ramosa* characters.

B.—Terminal and lateral inflorescence of F_2 plant, combining both *ramosa* and *tunicata* characters.

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PLATE 21

Pistillate inflorescence of F_2 plant, showing both the branched and tunicate characters.

EUPATORIUM AGERATOIDES, THE CAUSE OF TREMBLES[1]

By R. S. CURTIS, *Animal Husbandman, Beef-Cattle and Sheep Investigations*, and FREDERICK A. WOLF, *Botanist, North Carolina Agricultural Experiment Station*[2]

INTRODUCTION

In the mountainous sections of North Carolina considerable losses of domestic animals annually occur from a malady known as trembles. This disease is said to be transmissible to man through the ingestion of milk, certain milk products, or flesh of animals affected with trembles, and is known by physicians as milksickness. Since trembles is of so much economic importance to the live-stock interests of the State and since the investigations dealing with its etiology contain such diverse conclusions, a study of this disease was begun during 1916. It is deemed advisable to present at this time the considerable body of data which has accumulated relative to the relationship between *Eupatorium ageratoides* (Pl. 22) and trembles and to reserve for future investigation many important considerations which are as yet unknown or lack conclusive data. Data on certain other phases of the problem have already been secured, but a report of this part of the investigation is reserved for future publication.

HISTORICAL REVIEW

A very large number of papers dealing with milksickness, or trembles, have appeared in the hundred or more years during which the disease has been known. Most of these articles have been published in the various medical journals, and references to the most important of them are given in several of the more recent investigations (1, 2, 3, 4, 5, 6).[3] These recent papers, furthermore, contain a brief résumé of the important findings of the earlier students of this disease, so that it is necessary for the present purpose to review only the pertinent facts relative to the causes which have been regarded as productive of trembles. In general, these etiological factors may be placed in one of three groups—namely, mineral poisons, poisonous plants, and bacterial parasites.

Arsenic, lead, and cobalt are among the minerals which have been suspected of being the cause of the trouble, but these charges have been disproved.

Among the poisonous plants whose ingestion is regarded as the cause of the trouble are *Rhus toxicodendron*, *Eupatorium ageratoides*, *Lobelia inflata*, and *Bigelovia* (*Isocoma*) *heterophylla*. Moseley (4) was of the

[1] Published with the permission of the Director of the North Carolina Agricultural Experiment Station.

[2] Grateful acknowledgment is hereby made of the kindly assistance in this work of our colleagues Mr. Earl Hostetler and Dr. J. I. Handley.

[3] Reference is made by number to "Literature cited," p. 404.

opinion, from experiments in which *E. ageratoides* was fed to various animals, that this weed was the cause of the disorder. His results were not entirely convincing, however, and in criticism of them Crawford (1, p. 15) says:

> It can not be said that Moseley has even proved *Eupatorium ageratoides* to be a poisonous plant, much less the cause of "trembles."

When, in the summer of 1906, about 50 head of cattle died of trembles near Minooka, Ill., an investigation of *E. ageratoides* was undertaken by the Office of Poisonous Plant Investigations of the United States Department of Agriculture, since it was the popular belief that this weed was the cause of the trouble. Aqueous extracts were prepared from dried plants and plants preserved in water to which a small amount of chloroform had been added. These extracts were fed or injected subcutaneously into rabbits, cats, and dogs. An aqueous extract from the ash of dried plants was also administered to rabbits. Fifty-eight gm. of fresh plants were, furthermore, fed to a lamb weighing 25 kgm. without the production of symptoms of trembles. In summarizing the results of these experiments, Crawford says (1, p. 19–20):

> It certainly can not be said that it has been proved that milksickness is due to any constituent of *Eupatorium ageratoides*. * * *. Again severe epidemics have occurred in winter when the foliage has disappeared, which would tend to exclude the higher, nonevergreen plants as the cause of this disorder. In fact, all the evidence in hand is against the causation of this disease by such plants.

Subsequent publications by Moseley (5, 6) advance the claim that aluminium phosphate found to be present in leaves and stems of *E. ageratoides* is the active toxic principle. Animals fed on this weed or on food in which aluminium phosphate was mixed were found to void aluminium phosphate in the milk and urine. The blood of these animals and certain organs were, moreover, found to contain aluminium phosphate. The stems of rayless goldenrod (*Isocoma heterophylla*) were also found to contain aluminium phosphate; and when this weed, too, was fed to rabbits it produced symptoms similar to those following the feeding of *E. ageratoides* or aluminium phosphate. Since aluminium phosphate is insoluble in water, this accounts, as explained by Moseley, for Crawford's failure to produce poisoning in the experiments in which aqueous extracts of *E. ageratoides* were used. His criticism of the experiment in which 58 gm. of fresh weed were fed to a lamb weighing 25 kgm. is that this quantity would probably not be fatal to a full-grown rabbit.

Experiments conducted by Jordan and Harris (2, 3) in New Mexico and Texas, where this disease is present, but where *E. ageratoides* does not grow, indicate that the disease is of bacterial origin. Trembles developed in rabbits, guinea pigs, dogs, and calves by inoculation with a spore-forming organism which the authors described as *Bacillus lactimorbi*. This organism was present in the milk and butter of cows affected with trembles, in the feces of nonfatal cases in man, in certain parts of the

bodies of affected sheep and horses, and in the soil in regions where milksickness prevails. Taken as a whole, however, the experiments were far from decisive in showing that *B. lactimorbi* is the etiological factor in the production of trembles, or milksickness.

METHODS OF EXPERIMENTATION

Since *E. ageratoides*, commonly called "white snakeroot," does not grow in the vicinity of Raleigh, N. C., where the experiments were conducted, it was arranged to secure daily shipments of the green weed from Shooting Creek, N. C., where *E. ageratoides*, locally known as "richweed," grows luxuriantly, especially in shady situations. Since this place is about 300 miles distant from Raleigh, the weed used had been cut about 48 hours prior to its arrival at Raleigh. The weed was fed twice daily to sheep kept singly in small pens in a sheep barn. A maintenance ration of some dry concentrate was given in addition to this green food. The animals used were selected from the experimental flock of grade ewes, all of which were in a healthy condition and, with the exception of those used in the experiments, remained so. The flock number of each individual was retained and is used subsequently in reporting the experiments with the several animals. No case of trembles had ever appeared in this or any other of the Station flocks prior to or during the course of these experiments.

Each animal was weighed when it was placed on the experiment and at time of death. Beginning with experiment 3, in addition to the initial weighings subsequent weighings were made at 3-day intervals until the experiment was concluded or until death resulted. At first the grain and weed were fed separately; but, since the animals either avoided eating any of the weed or ate only sparingly of it, the weed was passed through an ensilage cutter and then mixed with grain before being fed. A daily account was kept of all of the food which was refused by each animal, and these data were employed in approximating the total amount consumed during the course of the experiment. The quantity of *E. ageratoides* eaten by each animal could only be approximated, since the weed and grain refused were mixed and since some loss of weight was due to desiccation. Post-mortem examinations were made of ewes 10, 11, 14, 27, 161, and 169. With the exception of ewe 169, the post-mortem examination showed no evidence that death resulted from any other cause than the feeding of *E. ageratoides*. Post-mortem examinations were not made of the other animals, because the external symptoms were clearly those which characterize trembles.

SYMPTOMS OF TREMBLES

Since the possibility exists that trembles in animals may develop from causes other than the feeding of *E. ageratoides* and that the symptoms may differ somewhat from those resulting from the ingestion of this

weed, a brief account of the symptoms observed in the experiments with sheep is pertinent. Considerable individual variation exists in the different animals, both in the period elapsing until the first symptom of trembles is apparent and in the period following until death ensues. Some were sick as early as three days after being placed on the experiment, and no effects were apparent for three weeks in other cases. Sheep usually live three or four days after the disorder is first noticed. Some remain alive, however, for nearly two weeks, and one animal characteristically affected entirely recovered. The feeding of *E. ageratoides* to this animal, however, was discontinued as soon as trembling was noted.

Sheep in the early stages of the disease are sluggish and lie quiet unless urged to rise. They may refuse to eat, or the appetite may be quite normal. There is generally a very considerable decrease in weight, most of which occurs in the last two or three days preceding death. Respirations are accelerated and somewhat labored. A marked stiffness of the legs and ataxia characterizes the movements in walking. If after a day or two the animal is made to stand for a few minutes or is driven a few yards, muscular spasm, especially in the limbs, is evident. The sheep then stands with hind limbs placed well under the body (Pl. 23, *A*) and all feet spread apart laterally. In this posture the back is bowed, the neck outstretched, and the head lowered. Within a few seconds the quivering spreads over the entire body, increases in intensity, and becomes a violent, involitional tremor (Pl. 23, *B*). This is accompanied by slight, intermittent, tetanic contractions of the musculature of the limbs. At this stage of trembling ataxia is very pronounced, and the animal is unable to stand (Pl. 24, *A*). It drops quickly into the normal resting posture (Pl. 24, *B*), whereupon the trembling immediately ceases. If the sheep is made to rise after it has lain down for a few moments, a second spasm of trembling ensues, with a repetition of the symptoms as described. Trembling may recur repeatedly every time the animal is made to stand. The quiescent period is shortened, however, after each spasm of trembling and may begin as soon as the animal is placed on its feet.

RESULTS OF EXPERIMENTS

EXPERIMENT 1.—Three ewes, No. 11, 26, and 10, were used in experiment 1, which was designed to determine whether harmful effects follow the feeding of *E. ageratoides*. This experiment was begun on June 17 and closed on August 2. However, from June 22 to July 6 and from July 18 to July 28 it was impossible to secure the weed. During these periods the animals were grazed on Bermuda grass pasture. Except during the two periods mentioned, a liberal supply of white snakeroot was fed just as it arrived from the point of shipment. In addition, a maintenance ration of grain was fed in a separate trough. Neither the weeds

nor the grain were weighed in this experiment. Initial and final weights of each animal were recorded.

In the period between June 17 and July 16 ewe 11 was fed on *E. ageratoides* and grain for an aggregate of 15 days. A typical case of trembles had developed by July 16, and death occurred two days later. Food was refused during these two days, and there was a decrease in weight from 102 to 77 pounds during the 29 days of intermittent feeding.

Ewe 26 was given a ration of snakeroot and grain for 22 days, between June 17 and August 2. No symptoms of trembles developed during this period. The initial weight of this animal was 91 pounds, and the weight at the time the experiment was discontinued was 74 pounds.

The control ewe, No. 10, was maintained on pasture alone from June 17 to July 28. On July 28 she was put in a pen and was given a ration of *E. ageratoides* and grain until her death, which occurred on August 2. This ewe trembled only slightly, was very weak and emaciated, and lost 20 pounds during the experiment. Although the symptoms were not as marked in this case as in ewe 11, yet all conditions indicated that death was due to trembles.

EXPERIMENT 2.—Three ewes, No. 14, 23, and 26, were employed in experiment 2. This experiment was planned to confirm the results secured in experiment 1. Since the animals used in experiment 1 had refused to eat any considerable quantity of snakeroot when it was fed separately, it was decided to pass the weed through an ensilage cutter and mix it with an equal quantity by weight of grain. One pound of this mixed feed was given each animal twice daily.

On the sixth day after ewe 14 was placed on the experiment she had developed trembles and died on the following day. Her initial weight was 80 pounds, and her weight at death was 73 pounds.

The first symptom of trembles in the case of ewe 23 was noted 19 days after the experiment was begun. A well-defined case of trembles developed in this animal, and she died 6 days after the first symptoms were noticed. Her weight when feeding was begun was 70 pounds, and there was a loss in weight of 8 pounds during the 25 days.

Since ewe 26 had shown no ill effects from the feeding of *E. ageratoides* in experiment 1, she was used in this experiment. It will be recalled that the weed was fed separately and was not ground in the first experiment. Ewe 26 had eaten only sparingly of the weed in this experiment. However, after 16 days' feeding with the mixed ration a very typical case of trembles developed. The feeding of the weed was therefore discontinued and she was put on pasture.

EXPERIMENT 3.—In this experiment ewes 12, 7, 29, 27, and 19 were fed the mixed ration to determine the amount of weed and the length of time required to develop trembles. Table I shows clearly the variation that exists with reference to these two points.

TABLE I.—*Results of feeding Eupatorium ageratoides to sheep—Experiment 3*

Ewe No.	Initial weight.	Experiment begun—	Feeding discontinued—	Days before death occurred.	Weight at death.	Feed consumed.	
						Grain.	Weed.
	Pounds.				*Pounds.*	*Pounds.*	*Pounds.*
12.....	74	Aug. 5	Aug. 18	[a]13	.....	5.5	11.5
7.....	86	...do.....	Sept. 1	27	61	13	9
29.....	81	...do.....	Aug. 21	16	55	8	10
27.....	70	...do.....	Aug. 10	5	56	2.25	4.5
19.....	113	...do.....	Aug. 23	18	89	8	11.25

[a] Feeding discontinued after 13 days.

Ewe 12 was taken off the experiment on August 18, at which time she was affected with trembles. She had lost only 1 pound during these 13 days. It will be noted that the amount of weed required to cause trembles in these five animals varied from 4½ to 11½ pounds and the range in time from 5 to 27 days.

EXPERIMENT 4.—In order to determine whether or not trembles is infectious, ewes 26 and 12 were put in a small Bermuda grass lot on August 18. Two healthy ewes from the flock were put in the same lot and all were fed grain in the same trough. It will be recalled that both ewe 26 and ewe 12 had typical cases of trembles when their feeding in experiments 2 and 3, respectively, was discontinued. Ewe 26 died on August 19 and ewe 12 still trembled a week afterwards. However, she finally recovered fully. Neither of the other two ewes had developed any symptom of trembles when the experiment was discontinued on September 4, and both subsequently remained normal.

EXPERIMENT 5.—This experiment was designed to determine the length of time that *E. ageratoides* must be fed to sheep when, after a certain number of days, the usual grain ration and pasturage are given. Two animals were therefore fed for three days on a mixture of equal parts of ground weed and grain and were then put on pasture. Two others were fed for six days before being placed on pasture and two others for nine days, after which they were put on pasture. Table II contains the essential facts in this experiment.

TABLE II.—*Results of feeding Eupatorium ageratoides to sheep—Experiment 5*

Ewe No.	Initial weight.	Final weight.	Days on experiment.	Feed consumed.	
				Grain.	Weed.
	Pounds.	*Pounds.*		*Pounds.*	*Pounds.*
169.....	83	78	3	1.5	1.5
171.....	85	80	3	1.5	1.5
162.....	96	82	6	5.5	5.5
168.....	89	79	6	4.25	4.25
161.....	102	62	9	6.75	6.75
170.....	105	92	9	5.5	5.5

Ill effects followed only in the cases of ewes 169 and 161, the former dying 8 days and the latter 11 days after being taken off the experiment. Since ewe 169 evidenced no marked symptoms of trembles, a post-mortem examination was made which showed that stomach worms (*Hemonchus contortus*) may have been a contributory cause of her death. Ewe 161, however, developed a typical case of trembles and is the animal represented in the accompanying illustrations (Pl. 23, 24).

EXPERIMENT 6.—Since certain sodium compounds have been suggested as antidotes for trembles, two sheep were given definite quantities of common stock salt and one baking soda along with the mixed ration of *E. ageratoides* and grain. This experiment was conducted between September 18 and October 20. The data on these three animals are presented in Table III.

TABLE III.—*Results of feeding Eupatorium ageratoides, together with salt or soda, to sheep.—Experiment 6*

Ewe No.	Initial weight.	Placed on feed—	Date of death.	Days before death resulted.	Weight at death.	Feed consumed.	
						Grain.	Weed.
	Pounds.				*Pounds.*	*Pounds.*	*Pounds.*
21.....	121	Sept. 18	Sept. 29	11	.....	8	5.25
28.....	96	...do.....	Oct. 7	19	73.5	15	10.25
37.....	118	Oct. 2	Oct. 20	18	78.5	10	6

Ewe 21 consumed 8 ounces of salt and ewe 37 ate 12 ounces during the periods of 11 and 18 days, respectively, in which they were on the experiment. Ewe 28 ate with her feed 30 ounces of baking soda.

EXPERIMENT 7.—Since it has been claimed that aluminium phosphate causes a disorder similar to that following the feeding of white snakeroot, two ewes, 166 and 167, were fed aluminium phosphate for a period extending from September 9 to November 17. During this time each ewe was fed 412 gm. of aluminium phosphate ($AlPO_4$; Baker's, C. P.) mixed with 68.5 pounds of grain, this being supplemented with 138 pounds of alfalfa hay. The daily amounts of aluminium phosphate given were gradually increased from 2 to 16 gm.

At no time during this period of 69 days were these ewes observed to manifest any symptoms of trembles. The initial weight of ewe 166 was 80 pounds, and her weight at the close of the experiment was 91 pounds. The initial and final weights of ewe 167 were 90 and 93 pounds, respectively.

SUMMARY

(1) *Eupatorium ageratcides*, commonly known as white snakeroot and locally known in North Carolina as richweed, had previously been claimed by Moseley to cause trembles in animals. This claim has been substantiated by experiments with sheep in which green plants of *E.*

ageratoides were fed during the months of June, July, August, September, and October, 1916.

(2) Fifteen cases of trembles in sheep have been developed from feeding *E. ageratoides*. Fourteen of these resulted fatally, and one of them recovered. Death of one of these sheep was probably due in part to an infestation of stomach worms.

(3) Death resulted in from 5 to 27 days following the beginning of feeding of *E. ageratoides*.

(4) Considerable variation existed in the several ewes, also, with reference to the quantity of weed ingested before trembles appeared.

(5) Indirect evidence against the infectious nature of the disease was secured by failure to communicate trembles from sheep characteristically affected to healthy sheep when they were confined and fed together in a small lot.

(6) Salt and soda in the amounts given along with a ration of grain and *E. ageratoides* were without apparent antidotal effect.

(7) No harmful effect followed the feeding for 69 days of aluminium phosphate mixed with grain and supplemented with alfalfa hay.

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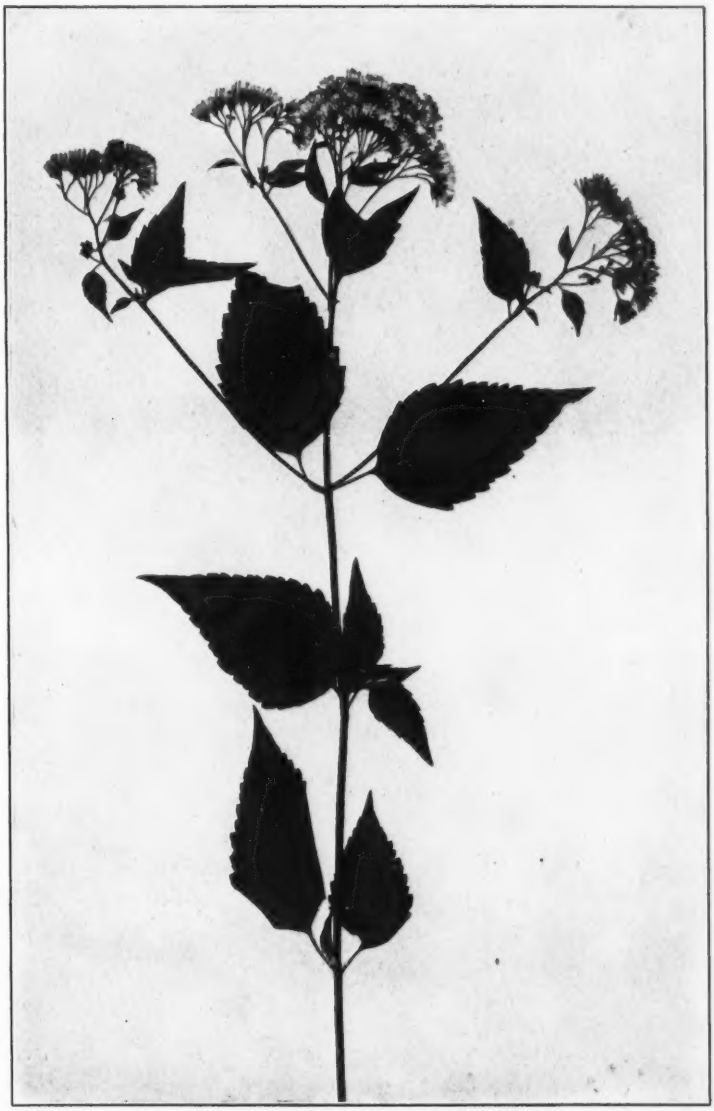
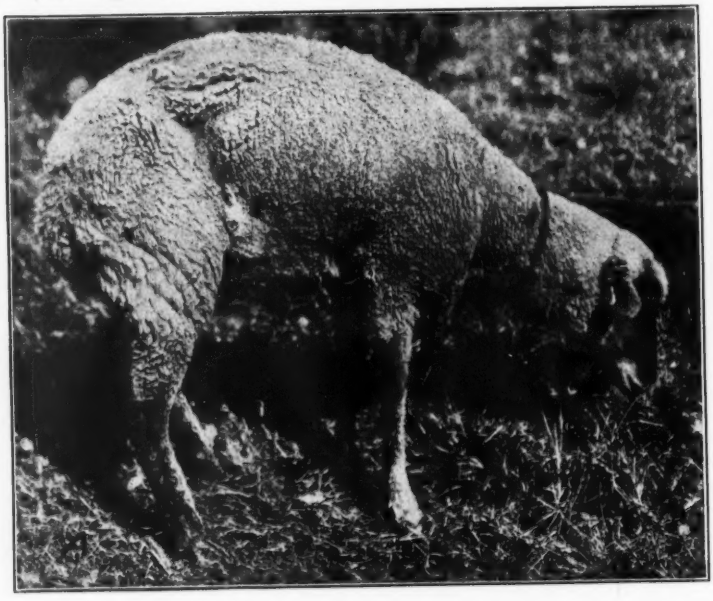
PLATE 22

Eupatorium ageratoides, or white snakeroot.





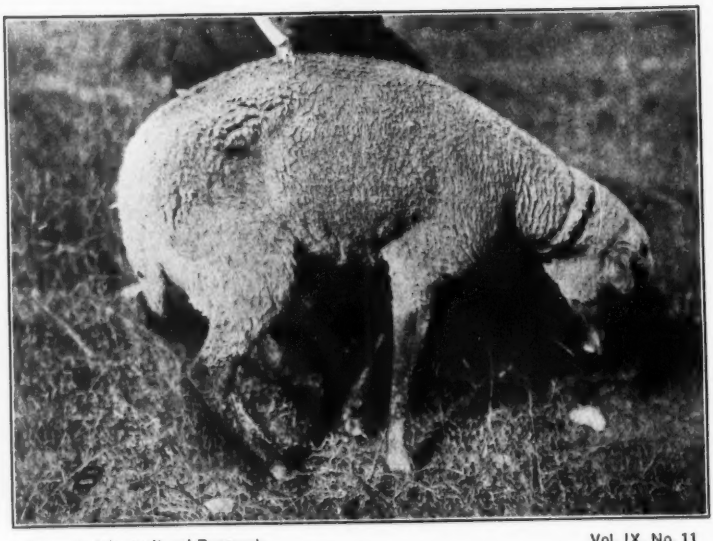


PLATE 23

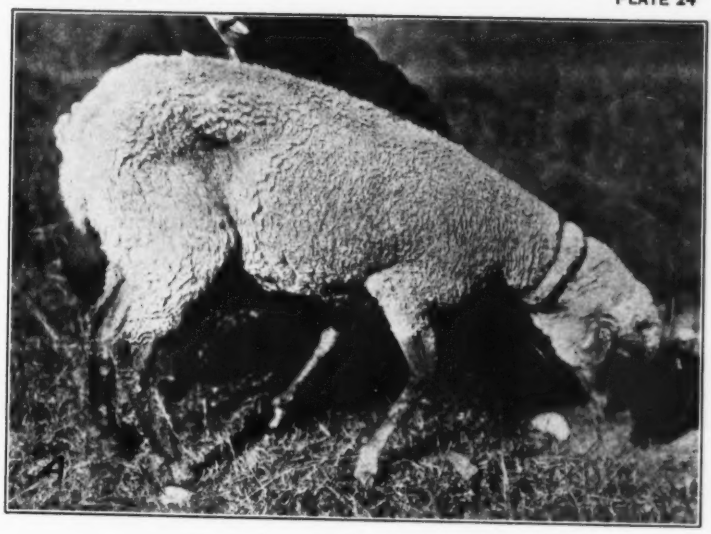
A.—Ewe 161 in the characteristic standing posture when trembling is quite violent.

B.—The same animal with feet spread apart in an effort to stand when the tremors have become more acute.

PLATE 24

A.—At this stage of trembling the animal is unable to stand and is beginning to drop.

B.—Ewe 161 in the position to which she has dropped after a violent spasm of trembling.


A

B